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INDUCED EFFECTS ON
THE EXOCRINE AND
ENDOCRINE RAT PANCREAS

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ENDOCRINE RAT PANCREAS

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by

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The present thesis is based on the following papers:

- I. B. Arnesjö, I. Ihse, I. Lundquist & I. Quist.
Effects on exocrine and endocrine rat pancreatic functions of bovine lung trypsin inhibitor administered perorally.
Scand.J.Gastroent. 1973, 8, 545-554.
- II. I. Ihse, B. Arnesjö & I. Lundquist.
Studies on the reversibility of oral trypsin inhibitor induced changes of rat pancreatic exocrine enzyme activity and insulin secretory capacity.
Scand.J.Gastroent. 1975, 10, 321-326.
- III. I. Ihse, B. Arnesjö & I. Lundquist.
Oral trypsin inhibitor induced improvement of exocrine and endocrine pancreatic functions in alloxan diabetic rats.
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- IV. I. Lundquist, I. Ihse & B. Arnesjö.
Carbohydrate metabolism in normal and diabetic rats following longterm oral trypsin inhibitor administration.
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- V. I. Ihse, B. Arnesjö & I. Lundquist.
Glucose-induced insulin secretion and pattern of exocrine pancreatic enzymes in the rat following oral and parenteral trypsin inhibitor administration.
Scand.J.Gastroent. 1974, 9, 719-724.
- VI. I. Ihse.
Abolishment of oral trypsin inhibitor stimulation of the exocrine rat pancreas following duodeno-jejunal resection.
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- VII. I. Ihse, B. Arnesjö & I. Lundquist.
Effects on exocrine and endocrine rat pancreas following longterm administration of CCK-PZ (cholecystokinin-pancreozymin) and synthetic octapeptide - CCK-PZ.
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The above articles will be referred in the text by their respective Roman numerals.

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INTRODUCTION

Management of patients with chronic inflammatory disease of the pancreas is most challenging for the gastroenterologist and the surgeon. In recent years, however, surgical procedures have been more common in the treatment of this disease (5, 18, 20, 41, 65). The proper application of direct procedures of ductal drainage has given the best results (5, 18).

The exocrine pancreatic insufficiency, which by definition always accompanies chronic pancreatitis (75), might partly be explained by an impaired secretion of the pancreatic juice into the intestine due to stenoses and calculae in the pancreatic duct. Hitherto, however, there is no convincing evidence of an improvement of the pancreatic function following surgical drainage procedures (46, 50, 65). This might be explained - at least partly - by the fact that in most cases the acinar tissue is too severely damaged to recover on its own. As several authors have suggested that the pancreas has the power of regeneration (19, 25, 80) a tool was sought by which it would be possible to stimulate remaining intact pancreatic tissue in patients with chronic pancreatitis who have undergone such drainage procedures, in the hope of initiating and favouring regeneration of pancreatic acinar cells.

It has been known since 1948 that feeding of raw soybeans to chickens results in pancreatic hypertrophy (11). The same effect has also been observed in rats (51, 52, 53). Furthermore it was found that the enlarged pancreases had an increased capacity to secrete pancreatic digestive enzymes (51, 52, 53, 72). Subsequently it was found that ingestion of a single meal of raw soybeans induced an immediate secretory response of the pancreas (52, 53). A heat-labile trypsin inhibitor discovered in raw soybeans by Ham and Sanstedt (34) and by Bowman (10) in 1944 was suggested as the cause of not only the pancreatic enlargement and the increased secretory capacity but also of the poor growth found in rats fed on raw soybean through interfering with the normal intestinal tryptic activity (61).

A reversal of the growth inhibition by supplementary feeding of essential amino acids was demonstrated by several authors. Pancreatic enlargement, however, remained unchanged (8, 9).

Stimulation of the exocrine pancreas has also been reported after feeding of other trypsin inhibitors such as lima bean inhibitor, ovomucoid inhibitor, bovine trypsin inhibitor and the synthetic inhibitor β -amino-benzamidine (β -ABA) (28, 59, 71, 79). The diverse origins of these different trypsin inhibitors, - their dissimilarity in molecular weight, heat lability, and other physicochemical properties - suggest that their physiological effect when fed to animals, might be related to their common property, their ability to inhibit tryptic digestion. The mechanism(s) by which oral trypsin inhibitors elicit their pancreatic stimulation is, however, not known. It has been suggested that the inhibitors

act by effectively removing trypsin from the intestine thereby preventing its supposed normal feedback regulation of the pancreatic secretion (31). Little is known as to how the enzymes accomplish this intra-intestinal control. It is possible, however, that trypsin in the intestine interferes with the release of a humoral factor, which acts a stimulus on the acinar cells of the pancreas. Removal (inhibition) of the enzyme from the intestine would then result in a change in the rate of the release of the humoral factor and an altered level of stimulation of the pancreatic enzyme secretion. Evidence for the existence of such a factor in the blood of rats fed on soybean trypsin inhibitor has been reported (42). The nature of the factor and the chain of events resulting in its release is not fully understood.

PURPOSE AND PLAN OF THE PRESENT INVESTIGATION

The present thesis constitutes a link in a series of studies aiming at the possible use of oral trypsin inhibitors as stimulants of the pancreatic function in patients with chronic pancreatitis operated with drainage procedures (5) in order to guarantee an unimpeded flow of pancreatic juice into the intestinal lumen. Before starting human studies, however, we found it necessary to perform a thorough study of the trypsin inhibitor effects not only on the exocrine but also on the endocrine rat pancreas primarily in order to discover possible noxious effects of the treatment. As bovine lung trypsin inhibitor, in contrast to other studied trypsin inhibitors, is known to inhibit effectively human trypsins (24) this substance was used in all our experiments. The reason for studying the endocrine function as well, was the increasing evidence for a functional relationship between endocrine and exocrine cells in the pancreas (6).

Another purpose of the studies was to elucidate further the possible mechanisms by which the trypsin inhibitors elicit their stimulation of the pancreas.

The choice of a suitable animal model for experimental pancreatitis is difficult because the pathogenesis of most forms of human pancreatitis is unknown. Whereas many methods of inducing experimental acute pancreatitis are known (13) no good model for the induction of experimental chronic pancreatitis has hitherto been reported. Therefore we have performed our experiments in normal rats with the exception of those experiments in which the trypsin inhibitor effects on experimental diabetes were investigated.

The potent sulphonylurea compound glibenclamide was supplied by Boehringer Mannheim, West Germany, and the β -adrenergic agonist L-IPNA (L-isopropylnoradrenaline) by Wintrop Products Co., England.

Commercial cholecystokinin-pancreozymin (CCK-PZ), extracted from hog mucosa, containing 1500 Ivy dog units per mg., was obtained in sterile ampoules from GIH laboratory, Karolinska Sjukhuset, Stockholm, Sweden. The synthetic C-terminal octapeptide of CCK-PZ was obtained from Dr. M.A. Ondetti, The Squibb Institute for Medical Research, New Brunswick, N.J., USA.

Alloxan Monohydrate was purchased from British Drug Houses Ltd., Poole, England.

METHODS.

Surgical procedure. All operations (small intestinal resection) were performed under light ether anesthesia with clean but not sterile instruments. About 30 cm of the proximal jejunum and the part of the duodenum located distally at the entrance of the bile duct were resected and a retrocolic end-to-end anastomosis (duodeno-jejunostomy) was performed. A single injection of 375 000 units of a mixture of benzyl- and procainpenicillin was given at the end of the operation. Furthermore, 5 ml of saline was instilled intraperitoneally and 5 ml of 5,5% glucose injected subcutaneously. When using this schedule the postoperative mortality was about 5%.

Experimental procedures. The trypsin inhibitor was in most experiments administered once daily via an orogastric tube. In one experiment the inhibitor was administered intraperitoneally once daily for 3 weeks (V) and in an other experiment commercial CCK-PZ or the synthetic octa peptide of CCK-PZ was administered subcutaneously three times daily for 10 days in doses corresponding to 2 Ivy dog units (VII).

Alloxan diabetes was induced by intravenous injection of 50 mg per kg body weight alloxan monohydrate.

The insulin secretory capacity was tested in unanaesthetized rats with glucose, glibenclamide or L-IPNA. Glucose was administered orally via an orogastric tube or, as with the two drugs mentioned above, intravenously via a tail vein.

Serial blood sampling was performed by orbital puncture according to Rerup and Lundquist (68).

Preparation of homogenates and extracts. Unless otherwise stated the animals were sacrificed 18 hours after the last trypsin inhibitor, CCK-PZ or water administration, by cervical dislocation and the pancreas was rapidly removed and freed from surrounding fat and connective tissue. Homogenization was performed in Ringer solution in a Sorvall Omnimixer. The homogenates were kept at -20°C until analyzed. The small intestine was emptied of its contents by rolling it with a glass cylinder. The intestinal aliquots were kept at -20°C until used.

In some experiments pancreatic insulin was extracted with acid alcohol (56) and the immunoreactive insulin determined according to Heding (36). These extractions were performed in pieces (50-100 mg) taken from the splenic part of the pancreas.

Biochemical assays. Protein was determined according to Lowry et al (48), as modified by Eggstein and Creutz (21). Lipase was assayed by a scaled-down version of the method described by Erlanson and Borgström using tributyrine as the substrate (22). Amylase was determined according to Dahlqvist (14) and trypsin activable trypsin activity according to Arnesjö and Grubb (4) using p -toluene sulphonyl-L-arginine methyl ester (TAME) as the substrate. Trypsin was estimated by a modified Hummel method using TAME as the substrate (39). As previously demonstrated by Arnesjö and Filipek-Wender (3) the enzyme activities were fully accessible for their substrates.

Determination of blood glucose was performed enzymatically (glucose oxidase) according to Marks (58). Radioimmunological determination of insulin in plasma or pancreatic extracts was performed according to Heding (36) using ^{125}I -labelled pig insulin and guinea-pig anti-pig-insulin. The tissue glycogen assays were performed by the method described by Rerup and Lundquist (69).

The *in vitro* studies of glucose utilization by isolated tissue were carried out as follows: liver, muscle (hemidiaphragms) and adipose tissue (epididymal fat pads) specimens were incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) supplied with 2 mg gelatine, 2 mg glucose and 0.1 μC (liver 0.3 μC) ^{14}C -glucose per ml of the incubation medium. The incubations were performed in a metabolic agitator (100 cycles/min) in an atmosphere of 95% O_2 and 5% CO_2 . The amount of labelled $^{14}\text{CO}_2$ formed was assayed according to Lyngsøe (55). ^{14}C -lipid was isolated according to Fain, Scow and Chernick (23). ^{14}C -glycogen in adipose tissue according to Leonards and Landau (47), ^{14}C -glycogen in liver

and muscle as described by Lundquist (57) and total glycogen content according to Rerup and Lundquist (69).

Calculations. The data obtained were subjected to statistical analysis using Student's t-test.

ORAL TRYPSIN INHIBITOR EFFECTS ON THE EXOCRINE PANCREAS

Other investigators have demonstrated pancreatic hypertrophy and increased pancreatic enzymic content and secretory capacity following longterm oral trypsin inhibitor administration to rats (11, 51, 52, 53, 72). The reports concerning the pattern of secretable enzymes within the enlarged pancreas are, however, somewhat conflicting (27, 29, 41, 44, 72, 73, 77) and to our knowledge the reversibility of the trypsin inhibitor induced pancreatic effects has not been studied. In our experiments the effects of longterm oral bovine lung trypsin inhibitor on the exocrine rat pancreas was tested with special reference to these problems.

One daily dose of 10 000 KIU of the bovine lung trypsin inhibitor caused an enlargement of the pancreas and an increased pancreatic protein concentration after 2 and after 3 weeks of treatment but not after 1 week (I). The specific trypsinogen activity (activity per mg protein) in pancreatic homogenates increased after 1, 2 and 3 weeks of treatment whereas the specific amylase activity was uninfluenced after 1 and after 2 but increased after 3 weeks and the specific lipase activity decreased significantly after 1 and after 2 weeks and was unchanged compared to the controls after 3 weeks (I).

On the basis of values obtained from a rat group sacrificed on the first day of the experiment it was possible to calculate the specific activities of the different enzymes within the pancreatic tissue mass increase after 1, 2 and 3 weeks of oral trypsin inhibitor treatment, respectively. In doing so it was found (Fig. 2) that the pancreatic trypsinogen seemed to change in a manner different from the amylase and lipase. This marked increase of the pancreatic trypsinogen was possibly attained at the expense of amylase and lipase in order to restore the intestinal trypsin concentrations to normal values (I). An inhibition of the intestinal rat trypsin by the lung bovine trypsin inhibitor was in fact demonstrated when estimating the intestinal trypsin 4 hours after the trypsin inhibitor ingestion (VI).

Studies with different doses of the inhibitor given for 3 weeks did not show any linear dose-response relationship although the wet weight

of pancreas, pancreatic protein and pancreatic enzyme increase was most pronounced when the highest dose - 100 000 KIU - was used. Therefore this dose was chosen in the subsequent experiments.

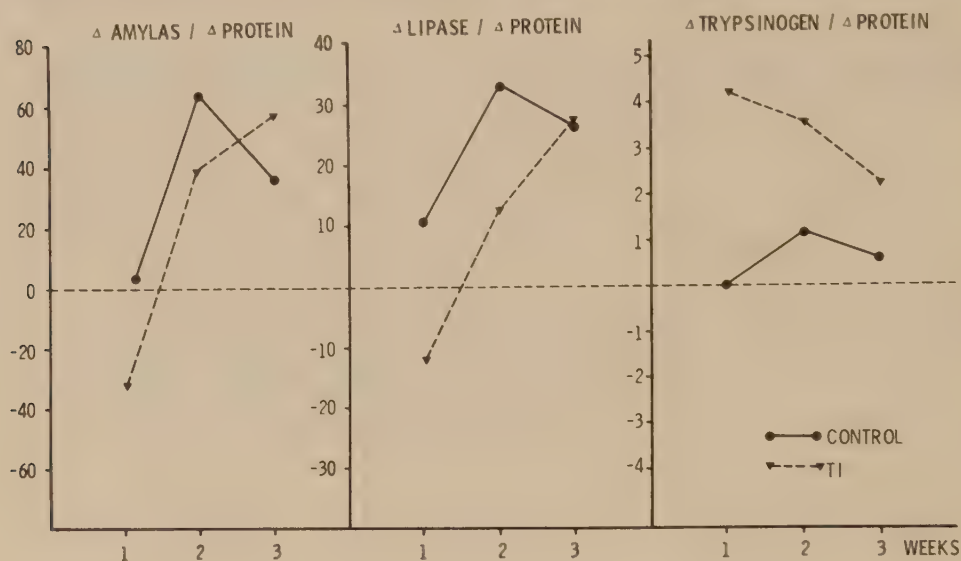


Fig 2.

Ratios between the change of pancreatic enzyme content (Δ -amylase, Δ -lipase, Δ -trypsinogen) and the corresponding change of protein content (Δ -protein) after ingestion of trypsin inhibitor for 1, 2 and 3 weeks (reprinted from paper I).

It has been suggested that different trypsin inhibitors when given orally to the rat have two distinct effects on the pancreatic function depending on the time factor (29). Ingestion of a single dose will induce an immediate secretory response of the pancreas resulting in a parallel release of enzymes whereas prolonged intake of the inhibitor will lead to enlargement of the gland and a preferential increase of its content of trypsinogen. As the secretagogue properties of other trypsin inhibitors already had been investigated in some detail (28, 31, 53, 72) we have only performed minor experiments in this field. As shown in fig. 3 A and 3 B the pancreatic amylase and trypsinogen decreased markedly within the pancreas 4 hours after the last trypsin inhibitor ingestion in a 3 week experiment whereas there was an increase of the intestinal amylase. The expected increase of the intestinal trypsin was not measurable until after 18 hours, possibly reflecting inactivation of trypsin by the intestinal inhibitor during at least the first 4 hours following the administration of the inhibitor. Thus, the bovine lung trypsin inhibitor, used by us, like for example the soybean trypsin inhibitor (53), egg-white trypsin inhibitor (72) and the synthetic trypsin inhibitor β -aminobenzamidine (28) seems to induce a secretory response from

the pancreas.

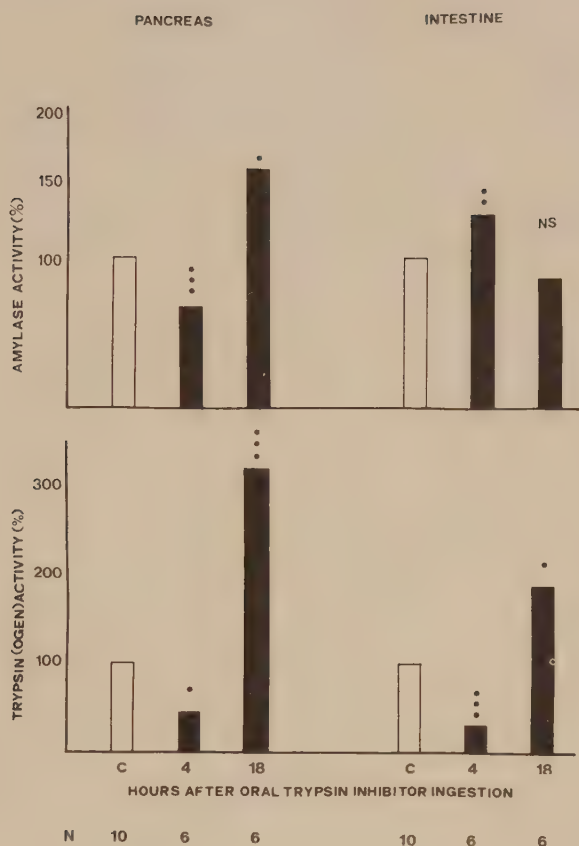


Fig. 3 A and 3 B. Pancreatic and intestinal enzyme activities (%) at different time intervals after oral trypsin inhibitor administration. * = $p < 0,05$, ** = $p < 0,01$, *** = $p < 0,001$. (Reprinted from paper VI).

In our longterm experiments - in which bovine trypsin inhibitor was used - the pancreatic trypsinogen always increased markedly (about + 150%) and on the whole the pancreatic amylase content increased (about + 30%). In some experiments there was also a slight increase of the pancreatic lipase after 3 weeks of treatment but often statistical significance was not reached (I, II, V, VI). The preferential increase of the pancreatic trypsinogen content found in these experiments thus

agrees with the results of other authors who used for example soybean inhibitor (44, 77). In repeated experiments an increase of intestinal trypsin was found when assayed 18 hours after the ingestion of the inhibitor. Intestinal amylase and lipase, however, remained unchanged compared to the control rats (VI). This finding might possibly point indirectly to a preferentially increased secretion of trypsinogen in comparison with amylase and lipase and thus agree with the results of Rothman and Wells that the trypsin inhibitors elicit a selective secretion of trypsinogen from the pancreas (72) rather than with the theory of a parallel trypsin inhibitor induced secretion of pancreatic enzymes advocated by, among others, Geratz (28). On the other hand, this finding might also reflect different times of disappearance from the intestine of the various enzymes.

As our studies aimed at a future therapeutic use of oral trypsin inhibitor in certain patients with chronic pancreatitis, we thought it necessary to study the reversibility of the effects observed in rats given the inhibitor (II). Therefore experiments were performed in which groups of rats were given the trypsin inhibitor by gastric intubation in a dose of 100 000 KIU daily for 3 or 8 weeks. In a third group the inhibitor treatment was interrupted after 3 weeks and thereafter the animals, like the controls, were given water in the same way once daily for another 5 weeks. After 3 and 8 weeks there was, as expected, a preferential increase of the pancreatic trypsinogen content and also a moderate increase of amylase while the lipase was unaffected. The wet weight of the pancreas increased after both 3 and 8 weeks while the pancreatic protein increased only after 3 weeks in this set of experiments. It was further found that 5 weeks after the cessation of a 3 week treatment the wet weight of pancreas, the pancreatic protein and the pancreatic amylase was found to be similar in treated as in untreated rats. The only remaining effect found in the "reversibility group" was an indisputable but moderate increase of the pancreatic trypsinogen (+ 61%). Thus, it seems legitimate to suggest that the trophic effects on the exocrine pancreas induced by oral trypsin inhibitor treatment are reversible - valuable knowledge before starting studies on human subjects (patients).

Diets containing trypsin inhibitors of different origin have been reported to have low nutritional value resulting in a retarded weight gain in mice, rats and chickens (35, 43, 63, 83). Several authors have proposed that the trypsin inhibitors cause poor growth presumably by virtue of their ability to inhibit intestinal proteolysis (61, 70). This hypothesis was further supported by the finding of a reversal of the growth inhibition by supplementary feeding of essential amino acids (8, 9). Lyman and Lepkovsky (52), on the other hand, assumed that the growth depression caused by trypsin inhibitors might be a result of an endogenous loss of proteins produced by a hyperactive pancreas. Furthermore other authors have reported failure of inhibitor - fed rats to ingest as much food as the controls (28) probably, however, not due to a repulsive taste as the same was observed after the feeding

of trypsin inhibitors of different origin. Impurities of the inhibitor preparations do not seem to be of importance as Geratz (28) when using the synthetic inhibitor β -aminobenzamidine recorded a decreased weight gain in inhibitor treated rats. In all our experiments the rats have been given bovine trypsin inhibitor in doses sufficient to induce enlargement of the pancreas. We have, however, never observed any retardation of the weight gain of the animals (I, II, V, VI). This might possibly be related to the different way we used to administer the inhibitor, i.e. one gastric intubation daily which, in contrast to inhibitor diets probably results in a decreased intestinal proteolysis only a few hours per day. In this context, however, it should be mentioned that Melmed and Bouchier (59) like us (unpublished) did not demonstrate any growth retardation when giving diets containing the bovine inhibitor to rats. Thus another possible explanation might be that this inhibitor for some reason lacks the property to induce growth retardation in rats although the stimulating effects on the pancreas are pronounced, pointing to different mechanisms for these two responses to the oral trypsin inhibitors.

In summary the results of the present studies show that one ingestion daily of bovine lung trypsin inhibitor to rats for several weeks induces an enlargement of the pancreas and an increase of the pancreatic protein concentration. After 1, 2, 3 or 8 weeks of treatment and even 5 weeks after the cessation of a 3 week treatment there was a marked increase of the pancreatic trypsinogen. During the first 2 weeks this increase of trypsinogen seemed to be attained on behalf of the pancreatic amylase and especially the pancreatic lipase synthesis. After 3 and 8 weeks of continuous treatment an increase of pancreatic amylase was observed whereas the lipase increased only in a few experiments. A corresponding increase of the intestinal trypsin and amylase indicated an increased secretion from the hypertrophied pancreas. Finally, contrary to other authors, we did not find any growth retardation of the rats in any of our experiments, possibly as a consequence of a different way of inhibitor administration in our experiments i.e. one gastric intubation daily.

ORAL TRYPSIN INHIBITOR EFFECT ON INSULIN SECRETION AND CARBOHYDRATE METABOLISM IN NORMAL RATS.

The most probable hypothesis regarding the mechanism of the effect of oral trypsin inhibitor ingestion on the pancreas favours the release of an intestinal factor or hormone as a consequence of the intraluminal trypsin inhibitor. As most known gastrointestinal hormones have been

proposed to influence the insulin release from the pancreatic beta cells (67), we found it of interest to study in the rat also the effect of the trypsin inhibitor treatment on the endocrine pancreas. Furthermore, recent investigations have shown some evidence of a functional relationship between exocrine and endocrine pancreatic cells. Thus exocrine pancreatic insufficiency often ensues diabetes mellitus (12, 82) and a high incidence of impaired insulin secretion has been reported in chronic pancreatitis (62). In addition insulin seems to be trophic to the peri-insular acinar cells and enhances the incorporation of amino acids in amylase (1). In the human intact exocrine pancreatic tissue is probably essential for elicitation of insulin release by gastrointestinal hormones (66).

In our early experiments we observed an impaired insulin response of the pancreas after intravenous glucose loads to rats treated for 3 weeks with oral bovine trypsin inhibitor (I). While, compared to the controls, a decrease of the insulin levels was found 3 and 7 minutes after intravenous glucose administration, decreased insulin values were not noticed until 30 and 60 minutes after orally administered glucose. The normal initial insulin secretory response observed after oral loads might be due to the release of one or more insulinotrophic gastrointestinal hormone(s) leading to a facilitated early insulin response in these rats. This suggestion is supported by the recent observations of Melmed, Turner and Holt (60), who found a normal insulin response to intravenous secretin or secretin-pancreozymin but decreased insulin response to intravenous glucose in soybean-fed rats. This was presumed by them to be related to an observed prominence of the so-called acinar- β -intermediate cells (vide infra). In our studies a decreased insulin release after 3 weeks of oral trypsin inhibitor treatment was also found after stimulation of the beta cells with other insulin secretagogues such as the potent sulphonylurea derivative glibenclamide or the β -adrenergic agonist L-isopropylnoradrenaline (II). As the insulin response to intravenous glucose was found to be normal after a treatment period of 1, 2, or 8 weeks and also 5 weeks after the cessation of a 3 week treatment, the impaired insulin response at 3 weeks seems to be a transitory as well as a reversible phenomenon (I, II).

Although the insulin release decreased after 3 weeks of treatment, the glucose tolerance was normal or even increased following intravenous and oral glucose loads (I, V). After 8 weeks and also 5 weeks after a 3 week treatment when the insulin response was normal, the glucose tolerance was found to be distinctly enhanced (II). Thus the improved glucose tolerance in treated animals seemed to be unrelated to the insulin secretory pattern.

Like us, Melmed et al (60) in 1973 reported lowered glucose-induced insulin secretion paradoxically associated with an enhanced glucose tolerance in rats given trypsin inhibitor diets (raw soybean) for 8-10 weeks. Melmed et al further reported a decrease of the pancreatic insulin content in treated rats. According to these authors this was

consistent with an observed reduction in islet size in soybean-fed rats. However, in our rats (treated with purified bovine lung trypsin inhibitor) the pancreatic insulin concentration was practically unaffected (II). Another observation made by Melmed et al was an increased prominence of the so-called acinar- β -intermediate cells. These cells which are exocrine acinar cells containing β -granules in addition to the zymogen granules might be of particular biological importance as their occurrence suggests a possible insulin-producing ability which might be different from that of the pure β -cell. According to Melmed et al these cells furthermore are selectively responsive to secretin-pancreozymin - an observation which possibly might reflect the normal early insulin response after oral glucose in our trypsin inhibitor treated rats (vide infra). Melmed et al conclude that the acinar- β -intermediate cells in certain species may represent a reserve capacity for insulin production which is only mobilized in time of need.

In order to find a possible explanation for the observed apparently non-insulin-dependent improvement of the glucose tolerance in trypsin inhibitor treated rats, studies of the carbohydrate metabolism in peripheral tissues were performed (IV). An increase of the glycogen concentration was found in liver and muscle after 3 weeks of treatment. Furthermore the glucose oxidation ($^{14}\text{CO}_2$ -production) was increased in muscle after 3 and 8 weeks and in liver after 3 weeks. An increase of the glycogen deposition was noticed in muscle tissue in rats treated for 3 weeks. In no respect any differences were found between treated and untreated rats in adipose tissue. It was concluded that the paradoxical increase of the glucose tolerance in trypsin inhibitor treated rats observed by us and by Melmed et al (60) at least to some extent might be a result of a facilitated glucose utilization in muscle and liver tissues. The mechanism which rules these peripheral effects of oral trypsin inhibitor treatment is not known. They might possibly be accomplished by a chronic release of and longterm influence on the tissues of one or more gastrointestinal factor(s) which does not seem to be identical with CCK-PZ, as longterm administration of impure CCK-PZ or of the C-terminal octapeptide of CCK-PZ did not induce changes in glucose utilization by muscle tissue (VII). That the observed changes do not seem to be the result of an acute effect on the tissues was further suggested by the finding that incubation of serum from trypsin inhibitor treated rats and muscle tissues from untreated rats did not cause any similar effect (IV).

In summary oral trypsin inhibitor administration to rats for 3 weeks appears to cause a transitory decrease of the insulin release after intravenous as well as oral glucose loads and after stimulation of the beta cells with glibenclamide or L-isopropylnoradrenaline. After 1, 2 and 8 weeks the insulin response was normal. Treated rats displayed an increased glucose tolerance which, however, was not related to the insulin secretory pattern. Instead the improved glucose tolerance possibly - at least partly - might be related to an observed improvement of

glucose oxidation and glycogen preservation in peripheral tissues, especially muscle. Thus the results might seem to legitimize studies on the effects of oral trypsin inhibitor administration in patients with chronic pancreatitis despite the observed transitory impairment of the insulin secretory capacity.

ORAL TRYPSIN INHIBITOR EFFECTS ON THE EXOCRINE PANCREAS,

INSULIN SECRETION, AND CARBOHYDRATE METABOLISM IN ALLOXAN

DIABETIC RATS.

Studies on insulin secretion and carbohydrate metabolism in trypsin inhibitor treated alloxan diabetic rats were performed as a consequence of the finding of an increased glucose tolerance in trypsin inhibitor treated normal rats (I, II, V). Due to the suggested functional relationship between endocrine and exocrine cells in the pancreas (6) the exocrine pancreas was also investigated in these experiments.

Diabetic rats chosen at random and treated with oral trypsin inhibitor displayed a more rapid weight gain than the diabetic controls. After 3 and after 5 weeks treated rats had lower basal glucose values than untreated. Furthermore, they displayed increased basal plasma insulin: blood glucose ratios and also increased pancreatic insulin content. These latter findings might perhaps be related to the increased prominence of the acinar- β -intermediate cells observed by Melmed et al (60) in rats fed on soybean trypsin inhibitor (vide infra). There was also a slight but significant increase of the insulin secretory capacity at intravenous glucose loads. In these severely diabetic rats no increase of the glucose elimination rate was found.

In inhibitor treated diabetic rats - *in vivo* - increase of the liver glycogen concentration was found after 3 and after 5 weeks. *In vitro* studies performed in muscle tissue did not reveal any increased glucose oxidation ($^{14}\text{CO}_2$ -production) in these severely diabetic rats, in contrast with the finding in normal rats. However, an augmented ^{14}C -incorporation into glycogen from ^{14}C -glucose was found in muscle after 3 and after 5 weeks of treatment. Thus a general improvement of glucose utilization capacity after inhibitor treatment was observed also in the diabetic rat.

In alloxan diabetic rats a marked decrease of the pancreatic and intestinal amylase was found. This is consistent with the results reported by Palla, Abdeljlil and Desnuelle (64) who in alloxan diabetic rats found a twentyfold reduction in pancreatic amylase which returned to normal with insulin treatment. Snook (76) also reported decreased pancreatic

amylase in a similar study. In his study, in which soybean trypsin inhibitor was fed to rats, no increase of the amylase activity was observed after the inhibitor treatment. Danielsson, noted that insulin in the mouse seems to play a role in maintaining a high level of amylase in the exocrine pancreas (15). Finally Söling and Unger found that insulin markedly enhanced the incorporation of labelled amino acids into rat pancreatic amylase *in vivo* (78). To our knowledge the role of insulin in the synthesis of other pancreatic enzymes has not been thoroughly studied. Our results (increase of the amylase in inhibitor treated rats having improved basal and glucose induced insulin secretory capacity) might further reflect a functional relationship between the endocrine and exocrine parts of the pancreas. In alloxan diabetic rats treated with oral bovine trypsin inhibitor the stimulation of the exocrine pancreas was found to be even more pronounced than in normal rats. Thus in diabetic rats, there was a marked increase of the pancreatic and intestinal lipase activities, a change seldomly seen in treated normal rats. A marked increase of the pancreatic and intestinal trypsin(ogen) and amylase was also found. The enlargement of the pancreas and the increase of the pancreatic protein concentration were pronounced. These results are in accordance with the findings of Snook (78) that the exocrine pancreas of alloxan diabetic rats seems to be more susceptible to trypsin inhibitor ingestion than that of normal rats. However, Snook in contrast to us, did not find any increase of amylase in inhibitor treated rats.

Patients with chronic pancreatitis sooner or later develop diabetes mellitus in about 30% of the cases. The results presented above - increased exocrine and endocrine pancreatic functions in experimentally induced diabetes - give further emphasis to the importance of testing this treatment in patients with chronic pancreatitis with and without ensuing diabetes.

In summary longterm oral bovine trypsin inhibitor administration to alloxan diabetic rats causes an even more pronounced stimulation of the exocrine pancreas than that found in normal rats. Treated rats had decreased basal blood glucose values compared to untreated. A slightly increased insulin secretory capacity was found in the basal state and after glucose loads. Furthermore, the total pancreatic insulin content was elevated. In these severely diabetic rats the glucose elimination rate did not improve as opposed to the findings in normal rats. The marked decrease of pancreatic and intestinal amylase demonstrated in alloxan diabetic rats seemed to be less pronounced in inhibitor treated rats (the latter had an increased insulin secretory capacity compared to untreated rats) possibly reflecting a role of insulin in the synthesis of amylase.

POSSIBLE MECHANISMS OF ORAL TRYPSIN INHIBITOR EFFECTS ON THE
RAT EXOCRINE PANCREAS, INSULIN SECRETION, AND CARBOHYDRATE
METABOLISM.

The mechanism whereby trypsin inhibitors lead to pancreatic enlargement with its attendant acceleration of enzyme synthesis is not known. It has recently been reported by Greene and Lyman that in the rat it is the level of active trypsin in the intestine that controls the amount of enzymes which are secreted from the pancreas. When the level of trypsin in the intestine drops markedly below normal, as would be the case in the presence of trypsin inhibitor, the pancreas responds by producing more enzymes. It was suggested that this effect is attained by the release from the intestinal wall of a humoral factor stimulating the exocrine pancreatic secretion (31).

Another hypothesis was presented by Laporte and Trémolières (45), who on the basis of acute experiments assumed that trypsin and trypsin inhibitors act through blood pathways after crossing the intestinal wall. These authors suggested that activity is the fundamental state of the pancreatic secretion and that this activity can be inhibited by trypsin in the blood. This inhibition on the other hand was thought to be suppressed by trypsin inhibitors in the blood. Laporte and Trémolières have also assumed that pancreasezyme acts by inhibiting trypsin (45).

Melmed and Bouchier (59) suggested that a primary function of the endogenously secreted pancreatic trypsin inhibitor may be to potentiate enzyme synthesis by the acinar cells, providing an important stimulus for the repletion of the digestive enzymes.

The results from our experiments strongly support the idea that the trypsin inhibitor effects on the pancreas require the mediation of the gastrointestinal tract. Thus we did not find any influence on the exocrine or the endocrine pancreas in rats following longterm parenteral in contrast to oral trypsin inhibitor administration (V). Furthermore there were no effects of oral trypsin inhibitor treatment on the exocrine pancreas in rats operated with a 30% proximal small intestinal resection (VI). This latter finding might reflect the removal of the site of production of one or more intestinal hormone(s) or factor(s) - responsible for the trypsin inhibitor effects - in resected animals. In this context it should be mentioned that all known intestinal hormones except enteroglucagon have been found in their greatest quantity in the proximal part of the small intestine (7). Results recently reported (34) have also indicated that trypsin does not exert its suppression of pancreatic enzyme secretion when introduced into the lower half of the small intestine.

The results of our studies of oral trypsin inhibitor effects on the endocrine pancreas suggest that a gastrointestinal factor or hormone is

released from the intestinal mucosa as a consequence of the intraluminal inhibitors. Thus we demonstrated in treated rats an increased glucose tolerance which apparently was unrelated to the insulin secretory capacity (I, II, V). This paradoxical increase of the glucose tolerance could partly be explained by an observed increase of glucose utilization and glycogen deposition in muscle and liver tissues (IV). It seems more likely that the favourable effects on carbohydrate metabolism are exerted by a longterm influence on the tissues by one or more gastrointestinal factor(s) than by an acute action since incubation of serum from trypsin inhibitor treated rats with muscle tissue from untreated rats did not cause any effects. Whether the trypsin inhibitor effects on the secretion of the unknown gastrointestinal factor(s) may be explained by an increased secretion of a factor which promotes glucose utilization or, alternatively, by a decreased secretion of a factor which normally inhibits glucose utilization remains to be elucidated.

Due to the potentiated secretion of zymogen enzymes within 30 minutes of ingesting a test meal containing purified trypsin inhibitor (53), coupled with the observation that the mean weight of the gallbladder of chickens fed on a diet containing soybean trypsin inhibitor was significantly less than that of the control group (74) several authors have suggested CCK-PZ as a possible mediator of the trypsin-inhibitor-induced pancreatic changes (31, 42, 59, 77). These observations imply that in addition to its gallbladder-emptying and zymogen-releasing properties, this hormone might have a specific trophic effect on the pancreatic acinar cell. In fact Rothman and Wells (71) in 1967 reported pancreatic enlargement after 3 subcutaneous injections of impure pancreozymin for 4 days to rats but not after injections of secretin. The pancreas of the pancreozymin-treated rats was found to contain relatively more chymotrypsinogen and amylase than trypsinogen. Snook (77) one year later reported increase of the pancreatic chymotrypsinogen, trypsinogen and lipase but not of amylase following 1 week of 3 daily subcutaneous injections of an impure CCK-PZ preparation. After 3 daily subcutaneous injections for 10 days of either an impure CCK-PZ preparation or of the synthetic octapeptide of CCK-PZ i.e. the active "exocrine site" of the CCK-PZ molecule (37), we found an enlargement of the rat pancreas and a parallel increase of amylase, lipase and trypsinogen in pancreatic tissue (VII). As the majority - but not all - of the studies on trypsin inhibitor effects on the exocrine pancreas speak in favour of a more marked increase of the pancreatic content of trypsinogen than of other enzymes, our results coupled with those of Rothman and Wells (71) and Snook (77), both using impure hormone preparations, do not fully support the idea that CCK-PZ is the mediator - at least not the sole mediator - of the trypsin inhibitor influences on the exocrine pancreas.

In our studies (VII) of rats treated with the octapeptide of CCK-PZ we did not find any discrepancies in blood glucose or insulin levels, compared to control rats, following an intravenous glucose load. In rats given the impure hormone preparation, however, we found decreased

insulin response and slightly improved glucose tolerance i.e. a pattern resembling that found after oral bovine trypsin inhibitor administration (I, II, V). These results might reflect the presence of other biologically active substances in the impure preparation and furthermore these observations do not favour CCK-PZ as the mediator of the effects on insulin secretion found by us in inhibitor-treated rats. In addition we could not demonstrate any changes in glucose utilization by muscle tissue in vitro either following the octapeptide or the impure CCK-PZ preparation. As already mentioned, rats given oral trypsin inhibitor were found to have a facilitated glucose utilization and increased glycogen deposition in muscle and liver tissues (IV).

As to the preferential production by the pancreas of one enzyme or one group of enzymes one can only speculate on the possibility of a neural or hormonal pathway. It has, however, been shown that the vagus nerves influence neither the acute secretory response nor the pancreatic enlargement induced by oral trypsin inhibitor treatment (53, 59). Therefore the most plausible answer seems to be a hormonal release from the intestine. This hypothetical agent might be a part of the mechanism that governs adaption of the pancreas to the type of food ingested i.e. production of a protein-rich secretion in response to a high-protein diet and formation of a juice rich in amylase during a high-carbohydrate regimen (32, 38). In this context it should be mentioned that Adelson and Rothman (2) recently isolated a peptide, chymodenin, from porcine duodenum which seems to elicit a specific increase in chymotrypsinogen secretion from the pancreas. This observation suggests that the regulation of digestive enzyme secretion may be enzyme-specific and raises the possibility that other peptides with analogous functions may exist. These results by Adelson and Rothman question the old concept of exocytosis i.e. a process which has been proposed as the mechanism of protein secretion (40) wherein the entire constellation of digestive hydrolases would be expelled in a parallel, en masse fashion after stimulus-induced fusion of the zymogen granule and the cell membranes forming an open channel to the acinar lumen. In addition recent studies by Dick and Felber (16) speak in favour of an intestinal hormonal system in which various food substrates specifically elicit the production of hormones that, through the blood stream, stimulate the release of pancreatic digestive enzymes specifically corresponding to the food intake.

In summary our results suggest that the effects of oral trypsin inhibitor treatment on the exocrine pancreas, insulin secretion and peripheral glucose utilization in fact are attained by regulation of hormonal release from the mucosa of the proximal small intestine. CCK-PZ does not appear to be the mediator - at least not the sole mediator - of these effects. Instead the action of still undiscovered biologically active intestinal factors must be considered.

CONCLUDING REMARKS.

The results of the present investigation show that longterm oral bovine lung trypsin inhibitor administration to normal rats causes an enlargement of the pancreas and an increase of the pancreatic protein concentration. The enlarged pancreas displayed increased enzyme content and an increased capacity to secrete digestive enzymes. In treated rats an improved glucose tolerance was found although the insulin secretory capacity was unchanged or even diminished. Furthermore an increased glucose utilization and glycogen deposition was found in muscle and liver tissues in these rats.

Also in diabetic rats the oral trypsin inhibitor treatment was found to result in a stimulation of the exocrine pancreatic function. Furthermore inhibitor treated diabetic rats had decreased basal blood glucose levels and slightly increased basal and glucose-induced insulin secretory capacity. The glucose elimination rate, however, was uninfluenced in these severely diabetic rats. An increased glucose utilization and glycogen deposition in peripheral tissues was found.

The results further suggest that the observed effects of oral trypsin inhibitor treatment are attained by the regulation of secretion of one or more intestinal hormone(s) from the proximal part of the small intestine acting upon pancreas and peripheral tissues.

According to the classification of pancreatic inflammatory disease adopted at the Symposium on Aetiology and Pathology of Pancreatitis, held in Marseilles 1963 (75), one cardinal symptom of chronic pancreatitis is a permanently decreased exocrine pancreatic function. Furthermore it is well-known that patients with this disease often suffer from diabetes mellitus. Therefore, if oral trypsin inhibitor treatment had similar effects in humans as those reported above, it could be of therapeutical benefit in certain cases of chronic pancreatitis by stimulating pancreatic function and possibly regeneration of acinar cells. Goldberg, Campbell and Roy studied the recovery of orally administered bovine trypsin and chymotrypsin in patients on ileal drainage (30). The enzyme recovery was calculated as the increment over the expected amount of enzymes (determined over four cosecutive days prior to the test) collected during a 48-hour period. With low doses the recovery was complete but with high doses of each enzyme a decreased recovery of chymotrypsin (38%) and suppression of the trypsin output, respectively, was demonstrated. These results are compatible with a feedback inhibition of the pancreas by especially intestinal trypsin. Trémolières noted in patients with pancreatic fistulas a cessation of the flow of the pancreatic juice after oral ingestion of high doses of lyophilized pancreas (81) further supporting the concept of a feedback regulation of intestinal trypsin on the pancreatic secretion. Based on our own observations and those of other authors (54) the inhibition of trypsin ought to be total or almost total in order to stimulate significantly the pancreas via the

feedback mechanism. Patients with chronic pancreatitis have decreased enzyme levels in the intestine but only in a proportion of the cases the decrease is so pronounced that steatorrhea occurs. It should be mentioned that steatorrhea and creatorrhea do not occur until 90% of the pancreatic function is destroyed (17, 49). Thus in one material we found steatorrhea in 7 out of 29 patients with histologically verified pancreatitis (5). This means that in patients with chronic pancreatitis the remaining functioning acinar cells of the pancreas might be stimulated after effective removal (inhibition) of their intestinal trypsin activity by orally administered trypsin inhibitors, especially if the flow of the juice to the intestine is secured by means of drainage procedures.

It has been shown by Figarella that human pancreatic juice contains two different trypsins (1 and 2) both of which are completely inhibited by the bovine trypsin inhibitor used by us. Soybean trypsin inhibitor, however, inhibits trypsin 1 to about 40% and trypsin 2 to about 80% while ovomucoid trypsin inhibitor is a still weaker inhibitor of the human trypsins (24). Thus the bovine inhibitor seems to be a suitable substance for human studies concerning the effect of oral trypsin inhibitor administration on the pancreas.

The main purpose of the present investigation was to pave the way for studies on the effect of oral trypsin inhibitor treatment in patients with chronic pancreatitis. The results presented suggest that such treatment might improve not only the exocrine pancreatic function but also the glucose utilization by peripheral tissues in both normal and diabetic rats. As, furthermore, no noxious effects were detected we find it legitimate to proceed with studies on the effects of oral trypsin inhibitor treatment in human subjects.

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